THE COUNCIL FOR TOBACCO RESEARCH - USA

110 East 59th Street

New York, New York 10022

STATUS PROGRESS REPORT VII

AUG. 1, 1974 to JULY 1, 1975

Title: The Effects of Fresh Cigarette Smoke Inhalation on the Respiratory Tract of Mice

Investigator: Clayton G. Loosli, M.D., Ph.D.
Hastings Professor of Medicine and Pathology
University of Southern California School of Medicine, 2025 Zonal Avenue, Hoffman Research Building, Room 915, Los Angeles, Calif. 90033

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Confidential: To Staff and Scientific Advisory Board Only

INTRODUCTION

The following is a brief summary of the status of our current studies being supported by the Council for Tobacco Research USA. Status progress report VI summarized studies up to August 1st, 1974. Studies which have been continued this past year will be summarized briefly.

Sendai Virus Infections: As you know, an extensive respiratory disease outbreak occurred in mice being subjected to cigarette smoke inhalation the summer and early fall of 1972. The etiological agent was identified as the Sendai virus. The surviving animals were continued to be exposed to cigarette smoke until the summer of 1973, when the animals were sacrificed, some having been exposed to cigarette smoke inhalation for nine months. The Air Pollution Building was then fumigated and new groups of animals were introduced in the late fall of 1973.

When the Sendai virus was isolated, it seemed important to set up experimental studies in order to compare the pathology of the spontaneous disease with that resulting from airborne exposure of the virus under experimental conditions. Several experiments were carried out. The pulmonary lesions were shown to be identical with those seen in the lungs of animals ill with the spontaneous illness. C57Bl/6J, C57L/J, SWR/J, and CD-1 strains of mice were equally susceptible to aerosolized Sendai virus. The lesions resemble very much those resulting from aerosol exposure of mice to the PR8-A influenza virus. Serological studies showed no cross reactions between the two myxoviruses.

Because of the tremendous cost, in time, animals, etc. of the spontaneous Sendai outbreak, it seemed important to see if subcutaneous vaccination with formalinized allantoic fluid culture of Sendai virus would protect against the experimental disease. Inactivated Sendai vaccine, given subcutaneously with and without Freund's adjuvant, to C57Bl/6J, C57L/J, SWR/J and CD-1 mice produced essentially complete protection against aerosols of virus containing several lethal airborne doses - prevention being against illness, death, and residual pulmonary lesions. One sub C dose of 0.1 to 0.15ml has been shown to be effective in preventing illness, death, and residual pulmonary lesions for 5 months. The data on the natural outbreak, experimental airborne infection, and protective effects of vaccination against Sendai virus infections, is being prepared for publication.

These studies support those recently reported by Fukumi and Takeuchi in Japan (Fukumi, H. and Takeuchi, Y: Vaccination against parainfluenza I virus (Typus muris) infection in order to eradicate the virus in colonies of laboratory animals. International Symposium on Immunity to Infections of the Respiratory System in Man and Animals. London, 1974. Developments in Biological Standardization, Vol. 28, pp. 477-481, Karger, Basel 1975. In this article, the seriousness of the problem in keeping SPF mice and guinea pigs free of Sendai virus infections is emphasized. They conclude that vaccination against the virus is efficient and the duration of immunity satisfactory.

Cigarette Smoke Inhalation Studies: This study began in late December, 1973 and January, 1974. At the time of onset of smoke exposure, the mice were 8 to 10 weeks old. Three strains of mice, C57Bl/6J, C57L/J, and SWR/J, all Sendai free, have been exposed to fresh cigarette smoke. Smaller groups of exposed and control (sham and shelf) mice have been sacrificed at 3,6,9, and 12 months after onset. Accurate records of the number of cigarettes to which the mice are exposed are being kept. Mice exposed to the smoke of one cigarette daily show little change, after one year, in lung structure, while those exposed to smoke from four cigarettes daily show epithelial hyperplasia, epithelial alveolarization, alveolar macrophage collections and chronic pneumonitis. No bronchial metaplasia or alveolar adenomas have been observed up to 12 months of smoke exposure. The C57Bl/6J show the least bronchial hyperplasia while the SWR/J show the greatest.

TABLE I

Cigarette Smoke Exposure Inhalation Schedule

Daily Exposure of Cigarettes Through 6-27-75

Mouse Strain	Age in Mo.	Duration in Mo.	4x Reg Diet	4x NoA Diet*	1x Reg Diet	Controls Sham Reg D	Controls Shelf Reg D
C57Bl/6J	22	20	56(1383)**	50(1350)	38(499)	25	28
C57L/J	22	20	46(1283)	48(1250)	50(373)	52	25
	TOTAL:		102	98	88	77	53
SWR/J Sacrificed 12th Through 17th, 1975							
SWR/J	21	19	18(1252)	28(1121)	38(328)	22	29

* Placed on NoA diet 2-10-75
** Total number of cigarettes per mouse

C57Bl/6J and C57L/J Mice: It is noted in Table I that mice in these two groups, as of the end of June, 1975, will be 22 months old and will have been exposed to smoke from 1A1 cigarettes for approximately 20 months. As of February 10th, 1975, half of the two groups were placed on a vitamin A deficient diet (NoA). As of now, 98 mice, exposed to smoke from 4 cigarettes daily, have been on a NoA diet for approximately 4½ months. As the mice in the above groups are tolerating the smoke exposure well, it is thought extremely important to continue to expose them for another four months or until they are at least 26 months old. Combining both NoA and regular diet groups, two hundred mice will then be exposed to cigarette smoke four times a day for approximately 24 months. As far as I know, now, there will be no other groups of mice in America which have been exposed to cigarette smoke as consistently and as long.

SWR/J Mice: Because the SWR/J smoke exposed mice are smaller in number (see Table I), they were sacrificed over a period of a week, from June 11th through the 17th. At the time of sacrifice, the mice were 21 months old and had been exposed to cigarette smoke for 19 months. As in the past, the lungs will be examined by light and electron microscopic, histochemical and biochemical (surfactant) procedures. Also, the livers of the smoke exposed groups on NoA and regular diets and of control animals will be titrated for vitamin A . The tissue specimens from the SWR/J mice will be processed immediately for examination.

Cigarette Smoke Inhalation Employing NoA and RD C57Bl Mice Followed by Influenza Virus Infection.

Mice 10 Months Old: One hundred-sixty (160) male C57Bl mice born October, 1974, were separated into two groups February 10th, 1975 and placed respectively on NoA and regular diets. An additional sixty mice of the same strain serve as controls. As of July 10th, these mice will be 9 months old and 80 will have been on a NoA diet for 5 months.

On July 14th these will be exposed to 1A1 cigarettes daily. The exposure will be gradually increased to, if possible, 6 cigarettes daily. The cigarette exposed mice, along with the sham and controls, will be housed in the Air Pollution Building. It is hoped that these mice can be exposed as per above schedule for 6 months or until December 30th, 1975. At this time, they will be subjected to sub-lethal clouds of influenza virus.

Smaller groups will then be sacrificed at increasing intervals of time. Comparative studies will then be made concerning the morphological and possibly the cancerous nature of the post-influenzal lesions as shown by light and electron microscopic, histochemical and biochemical procedures and subcutaneous transplantation of the post-influenza pulmonary nodules.

Mice 4 Months Old: A companion experiment to the above will be carried out using younger C57Bl/6J mice known to be vitamin A deficient as a result of having been on a vitamin A deficient diet since birth. Two hundred forty (240) mice will be employed, 120 on a regular diet and 120 on a NoA diet, plus 60 controls. After six months, they will be subjected to sublethal clouds of influenza virus, sacrificed and studied as above.

Influenza Virus Infections in C57Bl/6J Vitamin A Deficient (NoA) and Regular Diet (RD) Mice: Previous progress reports and two publications show that vitamin A deficient mice (CD-1 strain) show extensive hyalinization and keratinization of the regenerating bronchial membranes and post-influenzal epithelial nodules. This year, as has been stated, large numbers of vitamin A deficient C57Bl/6J mice have been raised by placing the pregnant females on a vitamin A deficient diet from 2 to 4 days before term and keeping the mother and offspring on the vitamin A deficient diet thereafter. After weaning, the mother is placed on a regular diet and after 6 weeks is mated again.

An experiment has just been completed and another one is in progress in which C57Bl/6J mice (NoA and RD) have been exposed to sublethal aerosols of mouse adapted influenza A viruses. As was observed in the lungs of CD-1 NoA mice, spectacular squamous metaplasia, characterized by extensive hyalinization and keratinization of the regenerating bronchial membranes and post-influenzal pulmonary nodules were seen. In the 18 and 25 day lesions, the lesions appear to be expanding (compressing surrounding lung tissue) and active. These resemble remarkably the illustrations of Nettesheim et al (J. Nat. Cancer Inst. 47: 697-701, 1971). The second experiment is similar to the first except that it will be carried on longer so that lesions from 40 to 80 days after onset can be studied. Some of the vitamin A deficient mice will be placed on a regular diet 30 days post infection to see if the squamous metaplasia and keratinization are reversed. Squamous cell post-influenzal nodules from some mice also will be transplanted subcutaneously to the same strain of NoA and RD C57Bl/6J mice. This study will continue through November, 1975.

Influenza PR8-A Virus Infection in NoA and RD C57Bl/6J Mice Previously Inoculated Intratracheally with Methylcholanthrene (MCA) and Benzo(a)pyrene (BaP): C57Bl/6J male and female mice raised on regular (RD) and vitamin A deficient diets (NoA) will be inoculated intratracheally after the method of Nettesheim et al (J. Nat. Cancer Inst. 47: 697-701, 1971).

BaP (Benzo(a)pyrene): The carcinogens will be suspended in sterile saline containing 0.20 percent gelatin. The animals will be anesthetized with IP pentobarbital and 0.3mg of BaP at six weekly intervals. Four weeks after the last injection, smaller groups will be subjected to sublethal clouds of influenza virus, along with NoA and RD mice not injected with carcinogens. This experiment is designed to compare the pathogenesis and pathology of the post-influenzal lesions in the different mouse groups and to determine if the influenza virus might act as a cocarcinogen.

This study will begin July 21st with at least 250 C57Bl/6J mice of the same age, half of which will be vitamin A deficient.

MCA (methylcholanthrene): 3-methylcholanthrene will also be given to separate groups of mice and studied as above.

SUMMARY

The above are brief outlines of experiments in progress which are designed to throw light on the role of(A) vitamin A deficiency in the production of lung changes in smoke exposed animals and (B) the possible co-carcinogenic effect of the PR8-A influenza virus in smoke exposed and carcinogen treated mice.

Respectfully submitted,

Clayton G. Loosli

Clayton G. Loosli, M.D., Ph.D.
Hastings Professor of
Medicine and Pathology

July 3rd, 1975.

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July 3rd, 1975

Justification of the Budget:

The above is a brief outline of studies in progress and supported by the Council for Tobacco Research - USA. These studies were referred to in progress report VI covering the period of February 1st, 1973 to August 1st, 1974. We estimate that it will take another nine months beyond October 1st, 1975 to complete them and to summarize and publish the data. We believe these studies are highly pertinent to understanding the effects of cigarette smoke inhalation on the lungs of experimental animals and, indirectly, of man.

While the principal investigator is three score and 10, the University Administration has extended the date of retirement in order for him to complete the above. The University's contribution in salaries is estimated to be twice that requested from the Council for Tobacco Research.

The salaries requested are only for the technical personnel. The smoke machine operators are for 6 months while the remaining are for 9 months. The material and supplies are based on the level of past expenditures during the past 3 years.

BUDGET

OCTOBER 1, 1975 to JUNE 30, 1976

A. Salaries

Professional:	TIME	SALARY	ANNUAL
Clayton G. Loosli, M.D., Ph.D. Principal Investigator	80%	0	0
Edwin B. Howard, D.V.M. Assoc. Professor of Pathology	20%	0	0
John Hardy, M.S. Research Assistant	100%	0	0
Virologist	50%	0	0
Secretary	80%	0	0
Technical:			
Daniel Popenoe, B.S., Histochemistry	50%	50%	*$3,000.00
LaVine Strom, B.S., Histologist	100%	100%	* 6,368.00
Raimonda Apeikis, M.S., E.M. Tech.	50%	50%	* 3,893.00
Carlos Garcia, Animal Caretaker	100%	100%	* 8,476.00
Alicia Navarro, Smoke exposure op.	100%	100%	** 3,946.00
Janna Saghatelian, Smoke exposure op.	100%	100%	** 3,645.00
			$29,328.00

*Nine months
**Six months

Fringe Benefits 12%:	3,519.00
SUB-TOTAL FOR A:	$32,847.00

B. Consumable Supplies (Major categories)

Vivaria	$1,500.00
Food - regular and vitamin A deficient	2,000.00
Fertile eggs	500.00
Histopath., histochem., microbiol.	500.00
Electron microscopy	500.00
SUB-TOTAL FOR B:	$5,000.00

C. Other Expenses (Itemize)

Service and repair of equipment	$1,000.00
Office and communication costs	1,000.00
Publication costs	2,400.00
Travel	600.00
SUB-TOTAL FOR C:	$5,000.00

RUNNING TOTAL OF A, B, C:	$42,847.00
INDIRECT COSTS (15% of ABC):	6,427.00
TOTAL:	$49,274.00

Respectfully submitted,

Clayton G. Loosli, M.D., Ph.D.

7-3-75